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Investigating Effect of Growth Promoting Bacteria and Nitrogen Fertilizer on Qualitative and Quantitative Traits of *Zea Mays* L. Hybrids

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ABSTRACT

Fertilizer management plays an important role in obtaining satisfactory yields from maize. In addition, fertilizer management is essential for achieving sustainable agriculture and protecting the environment. This research was conducted by split plot experiment based on randomized complete block design with four replications. Main factor was integrated with chemical and biological fertilizers in four levels that included 100, 75, 50, 25% quantities of nitrogen and phosphorus chemical fertilizers and with zero and 100% of biological fertilizers. Sub factor was three types of maize hybrids including Single cross 704, Single cross Karon 701 (SLD 45/1/2-1× MO17), Single cross Mobin (SLD 45/1/2-1× SLH 2/29/14/2-4/1). Analysis of variance indicated effect of fertilizer on all measured traits instead seed oil percentage and chlorophyll index was significant but effect of hybrids on all traits instead seed protein percentage was not significant. Interaction effect of treatments on seed yield, seed protein percentage and seed oil percentage was significant at 5% and 1% probability level, respectively. According result of mean comparison effect of different level of fertilizer treatment of 50% chemical fertilizer + 100% bio-fertilizer had highest amount of Biological yield (26.49 $t.ha^{-1}$), harvest index (56.71%), protein yield (1.19 $t.ha^{-1}$), oil yield (1.18 $t.ha^{-1}$), and chlorophyll index (53.04), although hybrid SC.704 by 50% Chemical fertilizer + 100% bio-fertilizer had higher seed yield (15.14 $t.ha^{-1}$), seed protein percentage (9.98%) and seed oil percentage (9.48%). Finally according result of this research use up biological fertilizers with half the recommended amount of chemical fertilizers is the greatest help towards sustainable agriculture.

Keywords: *Bio-fertilizers, Chlorophyll index, Oil, Yield.*

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INTRODUCTION

Maize is considered as one of the most important strategic and highly expected grains throughout the world. In order to have high quantitative and qualitative yield, maize must have an appropriate combination of nutrients (Malakouti and Gheibi, 2005). Chemical fertilizers are significant to succor nutrients in soil. Heavy doses of chemical fertilizers and pesticides are commonly used in order to enhance corn yields. Excessive nitrogen content in soil causes an inappropriate high uptake of this macronutrient by plants, which may result in inadequate growth and development due to the accumulation of nitrogen compounds in plant tissue (Szulc, 2013). In organic agriculture, one management goal is to increase and maintain soil quality with a high biological activity. Organic cropping system often has to deal with a scarcity of readily available nutrients in contrast to high input cropping system which relies widely available on soluble fertilizers (Soleimanzadeh and Ghooshchi, 2013). Nutrient management may be achieved by the involvement of organic sources, bio-fertilizers, and micro-nutrients (Singh *et al*., 2002). Indiscriminate use of chemical fertilizers to achieve high yield and to compensate for lack of nutrients and consequently the increase of production costs and destruction of soil and water resources have made the specialists interested in healthy and stable crop systems in terms of ecology (Tilak *et al*., 1992). More recently, a real challenge faces the workers in the agricultural research field to stop using the high rates of agro-chemicals which negatively affect human health and environment (El-Kholy *et al*., 2005; Kader *et al*., 2002). Farming practices which involve heavy application of chemical fertilizers may cause depletion of certain nutrients in soil and certain others would generally accumulate in excess resulting in nutrient imbalance which affects the soil productivity. Some of these problems can be tackled by using bio-fertilizers, which are natural, beneficial and ecologically friendly. Among the means available to achieve sustainability in agricultural production, organic manure and bio-fertilizer play an important and key role because they possesses many desirable soil properties and exerts beneficial effect on the soil physical, chemical and biological characteristics of the soil. His application of bio-fertilizers has become of great necessity to get a sufficient yield with high quality to avoid environmental pollution (Shevananda, 2008). Biological fertilizers are obviously an important part of a sustainable agricultural system and have an important role in crop production by maintaining soil fertility (Chen, 2006). Soleimanzadeh and Ghooshchi (2013) reported that high input cropping system was the most productive treatment but organic cropping system with bio-fertilizers was the most economical treatment with respect to increasing net profit. Combination mycorrhiza and bacteria holds promise for the organic cropping system of maize. Therefore in organic and low input cropping systems, a combination of mycorrhiza and free-living bacteria performed satisfactorily. According to Zeid (2008) a compound organic fertilizer and urea or a combination of urea and polyamines significantly enhanced yield, growth, and the chlorophyll index. Jafari Haghighi and Yarmahmodi (2011) in conclusion for reach to high yield in corn stated biological fertilizer cannot sufficient but integrated application of fertilizers (Biological and chemical fertilizers) became causes significant increase in yield. Use of bio-fertilizers offers agronomic and environmental benefits to intensive farming systems in Egypt, and the data showed that using *Azospirillum brasilense* or commercial bio fertilizers in cereals with a half nitrogen rate (144 $kgN.ha^{-1}$) caused a significant increase in yield. Further, seed inoculation with Rhizobium, phosphorus solubilizing bacteria, and organic amendment increased the seed production of the crop (Panwar *et al*., 2006). Yazdani *et al*. (2009) demonstrated that use of growth stimulating bacteria and phosphate solute in combination with chemical fertilizer was able to reduce phosphorus fertilizer application by 50% without an occurrence of reduced corn yield. Nitrogen is a basic plant component, playing a decisive role in the intensification of plant production (Scharf, 2002). Nitrogen being the major constituent of chlorophyll therefore increases in nitrogen availability leads to increase in chlorophyll content. In the chemical treatments, nitrogen is supplied more quickly and chlorophyll synthesis proceeds rapidly while in organic treatment nitrogen release slowly and supply required nitrogen during time. The significant differences between chemical and organic (whether full organic whether integrated) treatments may be attributed to the higher levels of nutrients besides growth stimulating substances (Enzymes, antibiotics and growth hormones) available in vermicompost (Vadiraj *et al*., 1998). Diverse maize genotypes i.e., single cross and double cross hybrids, synthetics, and composites, are being grown. These genotypes respond differently to various agro management practices, especially plant density and nutrition management (Nouraki *et al*., 2016). This study was designed to investigate the effects of applications of biological and chemical fertilizer on quantitative and qualitative traits of corn hybrids under warm and dry climate condition.

MATERIALS AND METHODS

Field and Treatment Information

This research was conducted by split plot experiment based on Randomized Complete Block Designs (RCBD) with four replications at Experimental Field in Shoushtar region at southwestern of Iran (Latitude 32 30' N and longitude 48 20' E and 18 meters above sea level) with moderate winters and hot summers in 2013. Main factor integrated of biological and chemical fertilizers in four levels and sub factor consisted of types of maize hybrids in three levels. Hybrids included Single cross 704, Single cross 701 (SLD45/1/2-1× MO17), Single cross Mobin (SLD 45/1/2-1× SLH 2/29/14/2-4/1). Chemical fertilizers included urea (46%), Triple superphosphate (46%) and Potassium sulphate (50%) and biological fertilizers included Nitroxin (Including *Azospirillum* sp., *Pseudomonas* sp., *Azotobacter* sp.) and bio-super phosphate microbial biofertilizer including *(Bacilus caogulans*) that this was applied in the form of seed inoculation and fertigation. Biological fertilizer was applied together with the irrigation water at the eight leaf stage. The experiment comprised of the following treatments:

1. Chemical fertilizer (100%) +non biological fertilizer + single cross 704
2. Chemical fertilizer (100%) +non biological fertilizer + single cross Mobin
3. Chemical fertilizer (100%) +non biological fertilizer + single cross701 (Karon)
4. Chemical fertilizer (75%) +biological fertilizer (100%) + single cross 704
5. Chemical fertilizer (75%) +biological fertilizer (100%) + single cross Mobin
6. Chemical fertilizer (75%) + biological fertilizer (100%) + single cross 701 (Karon) **7.** Chemical fertilizer (50%) + biological fertilizer (100%) + single cross 704

8. Chemical fertilizer (50%) +biological fertilizer (100%) + single cross Mobin
9. Chemical fertilizer (50%) +biological fertilizer (100%) + single cross 701 (Karon)
10. Chemical fertilizer (25%) + biological fertilizer (100%) + single cross 704
11. Chemical fertilizer (25%) + biological fertilizer (100%) + single cross Mobin
12. Chemical fertilizer (25%) + biological fertilizer (100%) + single cross 701 (Karon).

There were 12 treatments per blocks. The experiment was row to row distance of 75 cm, each treatment having six rows with a length of 5 m. Distance of seeds inter row was 18 cm.

Crop Management

Soils were fertilized according to recommendation based on soil tests (Table 1) and the level of treatments. The field was plowed, fertilized, and leveled before the field maize planted. The size of each plot was 6×5 m² and each block has 12 treatments. For the experiment, the distance between rows to rows was 75 cm with six rows per treatment, and irrigation was applied when the plants required it. The size of each plot was 6×5 m² and each block has 12 treatments. For the experiment, the distance between rows to rows was 75 cm with six rows per treatment, and irrigation was applied when the plants required it. Phosphorus and potassium fertilizers were provided from 150 kg.ha^{-1} triple superphosphate and 150 kg.ha^{-1} potassium sulfate. Biological fertilizer of nitroxin was used as much as two L.ha^{-1} as combined with seeds. Nitrogen chemical fertilizer was provided from the urea source, 50% during planting and 50% during 8-leaf stage. Irrigation was done every three or four days and after the plant establishment it was done every seven to ten days if necessary. Weeds were controlled via Cruise herbicide by two L.ha^{-1} at 4-to-5-leaf stage and Krakrown pesticide by one L.ha^{-1} against leaf and stem borer larvae.

Table 1. Physical and chemical properties of the experiment field

Soil depth (cm)	**0-30**	**30-60**
Soil Acidity (pH)	8.44	8.51
Electrical conductivity (ds.m^{-1})	4.07	2.69
Organic carbon (%)	0.51	0.36
Absorbable Phosphorus (ppm)	8	7
Absorbable potassium (ppm)	181	171
Clay (%)	26	24
Silt (%)	41	40
Sand (%)	33	32
Soil texture	Loam	Loam

Traits Measure

The studied traits included seed yield, biological yield, and harvest index, seeds per unit of area, protein percent, protein yield, oil percent, oil yield and Chlorophyll index. Total dry matter and seed yield were estimated after the physiological maturity. The samples were dried for 48 hours in the oven at 72-75 $^{\circ}$C and dry weight was measured. The number of seeds per unit of area obtained from multiplying the number of plants per unit of area and number of seeds per ear. To measure the seed protein percentage, Kjeldahl method was used (Page *et al.*, 1982; Bremner and Breitenbeck, 1983). Protein yield obtained from multiplying seed yield by protein percentage. Also to measure the oil percentage, Soxhlet method was used and oil yield obtained from multiplying seed yield by oil percentage (Cox and Cherney, 2005). In order to measure Chlorophyll index of leaves, from three points of leaf measured chlorophyll with SPAD 502 device and the average of three numbers was considered.

Statistical Analysis

The analysis of variance was done by SAS software (Ver 9.1) and the means were compared using Duncan's multi range test at 5% probability level.

RESULTS AND DISCUSSION

Biological yield

According the result of analysis of variance effect of different fertilizer treatments on biological yield was significant at 1% probability level, but the differences between hybrids and interaction effect of treatments was not significant (Table 2). Mean comparison result reveled the highest and the lowest biological yield was belonged to 50% chemical fertilizer + 100% bio-fertilizer (26.49 $t.ha^{-1}$) and 100% chemical fertilizer treatments (22.43 $t.ha^{-1}$), respectively (Table 3). Nouraki *et al*. (2016) and Naserirad *et al*. (2011) reported same result. Bio-fertilizers by increasing nitrogen the efficiency and uptake cause most shoot growth and consequently increasing the biological yield. Other reports have indicated that seed inoculation of corn with plant promoting bacteria in addition to 30 to 35% reduction of nitrogen fertilizer improved plant growth. Increased microbial biomass is directly related to soil health; it enhances the balance of nutrient elements and nutrient availability in root rhizosphers that promotes growth and ultimately affects a higher yield (Biari *et al*. 2008; Boddey *et al*., 1988).

Seed yield

Analysis of variance indicated the effect of different fertilizer, hybrids and interaction effect of treatments on seed yield was significant at 5% probability level (Table 2). According result of mean comparison the highest and the lowest seed yield was belonged to SC.704 hybrid with 50% chemical fertilizer + 100% bio-fertilizer (15.14 $t.ha^{-1}$) and Mobin hybrid with 100% chemical fertilizer (11.00 $t.ha^{-1}$), respectively (Table 4). In all three hybrids, nitrogen consumption reducing by 50%, and using bio-fertilizer increased seed yield. Biari *et al*. (2008) and Gholami *et al*. (1999) confirmed that result. Nouraki *et al*. (2016) reported mixing of biological fertilizers with chemical fertilizers could reduce the needs of chemical fertilizers up to 25% and these results are comparable to the application of 100% chemical fertilizers. Therefore, the best hybrid maize is the single cross 704 that has good yield potential when the chemical fertilizer is used at either 25% or 50% of the current application when mixed with bio-fertilizer. Other studies determined that plant growth was improved even when the nitrogen fertilizer applied was reduced by 30-35% as long as the seeds had been inoculated with growth promoting bacteria. An increase in the biomass of the microbial community was related to the soil health as this had an effect on the balance and availability of nutrients in the rhizosphere of the roots that lead to a higher yield (Boddey and Dobereiner, 1988; El-kholy *et al*., 2005; Biari *et al*., 2008).

Harvest index

The physiological ability of a hybrid to convert total dry matter in to grain yield is determined by its harvest index (HI). Analysis of variance showed that effect of fertilizer treatment on harvest index was significant at 1% probability level, but the difference between hybrids and interaction effect of treatments were not significant (Table 2). Mean comparison indicated that the highest and the lowest amount of harvest index belonged to 50% chemical fertilizer + 100% bio-fertilizer (56.71%) and 25% chemical fertilizer + 100% bio-fertilizer (48.31%) (Table 3).

Table 2. Analysis of variance of measured traits

S.O.V	df	Biological yield	Seed yield	Harvest index	Seed protein percentage	Protein yield	Seed oil percentage	Oil yield	Chlorophyll index
Replication	3	0.87^{ns}	0.25^{ns}	10.22^{ns}	0.25^{ns}	0.005^{ns}	1.17^{ns}	0.02^{ns}	117.67^{ns}
Fertilizer (F)	3	41.63^{**}	11.48^{*}	164.17^{**}	1.99^{*}	0.16^{*}	1.08^{ns}	0.12^{*}	34.07^{*}
Error I	9	8.37	1.31	54.57	0.37	0.02	1.01	0.04	29.46
Hybrid (H)	2	15.71^{ns}	6.77^{*}	3.65^{ns}	2.02^{*}	0.11^{ns}	0.19^{ns}	0.02^{ns}	36.77^{ns}
F × H	6	10.84^{ns}	4.99^{*}	25.70^{ns}	3.77^{*}	0.07^{ns}	1.90^{**}	0.04^{ns}	51.44^{ns}
Error II	24	7.46	2.17	33.77	0.48	0.02	0.31	0.01	42.17
CV (%)		4.17	2.29	5.82	6.7	6.26	5.90	4.62	6.64

ns, * and ** : non-significant, significant at the 5% and 1% probability level, respectively

Table 3. Mean comparison effect of different amounts of biological and chemical fertilizer on measured traits via Duncan test

Treatments	Biological yield ($t.ha^{-1}$)	Harvest index (%)	Protein yield ($t.ha^{-1}$)	Oil yield ($t.ha^{-1}$)	Chlorophyll index
100% chemical fertilizer	22.43^{*c}	55.10^{b}	1.03^{b}	1.01^{b}	52.05^{b}
75% chemical fertilizer + bio-fertilizer	23.43^{bc}	54.69^{b}	1.05^{b}	1.09^{b}	51.20^{b}
50% chemical fertilizer + bio-fertilizer	26.49^{a}	56.71^{a}	1.19^{a}	1.18^{a}	53.04^{a}
25% chemical fertilizer + bio-fertilizer	25.50^{b}	48.31^{c}	0.91^{c}	0.95^{c}	49.09^{c}

*: In each column means have similar letters do not have significant difference at 5% probability level.

Table 4. Interaction effect of treatments on measured traits via Duncan test

Treatments		Seed yield ($t.ha^{-1}$)	Seed protein percentage (%)	Seed oil percentage (%)
Fertilizer	**Hybrid**			
100% Chemical fertilizer	SC.704	13.45^{*ab}	7.18^{bc}	7.51^{cd}
	Mobin	11.00^{d}	9.06^{ab}	8.86^{ab}
	Karon	13.77^{ab}	8.21^{ab}	7.64^{bc}
75% Chemical fertilizer + bio-fertilizer	SC.704	11.28^{cd}	8.59^{ab}	8.20^{ab}
	Mobin	13.34^{b}	7.89^{bc}	8.19^{ab}
	Karon	13.81^{ab}	8.26^{ab}	8.99^{ab}
50% Chemical fertilizer + bio-fertilizer	SC.704	15.14^{a}	9.98^{a}	9.48^{a}
	Mobin	13.41^{ab}	7.81^{b}	8.67^{ab}
	Karon	14.84^{ab}	7.96^{bc}	7.46^{c}
25% Chemical fertilizer + bio-fertilizer	SC.704	12.98^{c}	8.84^{b}	7.96^{bc}
	Mobin	11.72^{cd}	6.09^{c}	6.28^{d}
	Karon	11.91^{cd}	7.31^{bc}	8.17^{b}

*: In each column means which have similar letters do not have significant difference at 5% probability level.

The results were similar to findings of another researchers (Zahir *et al.* 1998; Soleimanzadeh, and Ghooshchi, 2013). Increase of harvest index due to the increase of nitrogen fertilizer in maize can physiologically attribute to the increase of leaf area continuity and, nitrogen availability. In fact by creating balance between the nutrients bio-fertilizers increase both vegetative and reproductive growth and by creating adequate destination (seed), the assimilates will mobilize into seeds and ultimately the harvest index of plant seed increase (Araei *et al.*, 2014).

Seed protein percentage

Result analysis of variance revealed that the effect of different level of fertilizer, hybrids and interaction effect of treatments on the protein percentage was significant at 5% probability level (Table 2). Mean comparison indicated that the highest and the lowest protein percentage belonged to SC.704 hybrid with 50% chemical fertilizer (9.98%) and Mobin hybrid by 25% chemical fertilizer with 100% bio-fertilizer (6.09%) (Table 4). The results were in similar with the finding of other researchers (Chen, 2006; El-Kholy *et al*., 2005). Increase protein percentage with using bio-fertilizers is due to the effect of bacterial inoculation that increased the effective regulation of the growth, physiological and metabolic activity of the plant (Eidy Zadeh *et al*., 2012).

Protein yield

According result of analysis of variance the effect of different level of fertilizer on protein yield was significant at 5% probability level, but different hybrids and interaction effect of treatments was not significant (Table 2). Mean comparison result showed the highest and the lowest Protein yield was belonged to 50% chemical fertilizer+ 100% bio-fertilizer (1.19 $t.ha^{-1}$) and 25% chemical fertilizer+ 100% bio-fertilizer (0.91 $t.ha^{-1}$) (Table 3). The result was similar to finding of Rizwan *et al*. (2008).

Seed oil percentage

Result analysis of variance showed that the effect of different level of fertilizer and hybrids on seed oil percentage was not significant but interaction effect of treatments was significant at 5% probability level (Table 2). Mean comparison result revealed that the maximum and the minimum seed oil percentage belonged to SC.704 hybrid by 50% chemical fertilizer and 100% bio-fertilizer (9.48%) and Mobin hybrid by 25% chemical fertilizer with the 100% bio-fertilizer (6.28%) (Table 4).

Table 5. Correlation coefficient between measured traits

Traits	Seed yield	Biological yield	Harvest index	Protein percentage	Protein yield	Oil percentage	Oil yield	Chlorophyll index
Seed yield	1							
Biological yield	0.71^{**}	1						
Harvest index	0.69^{*}	-0.62^{*}	1					
Protein percentage	0.14^{ns}	-0.08^{ns}	0.38^{ns}	1				
Protein yield	0.51^{*}	0.55^{*}	0.58^{*}	0.73^{**}	1			
Oil percentage	0.11^{ns}	-0.33^{ns}	0.28^{ns}	-0.58^{*}	0.29^{ns}	1		
Oil yield	0.55^{*}	0.42^{*}	0.61^{*}	0.45^{ns}	0.83^{**}	0.52^{ns}	1	
Chlorophyll index	0.02^{ns}	-0.05^{ns}	0.00^{ns}	-0.44^{ns}	-0.26^{ns}	-0.27^{ns}	-0.14^{ns}	1

^{ns}, *, **: non-significant, significant at 5 and 1 % probability level, respectively.

Oil yield

According result of analysis of variance the effect of different level of fertilizer on oil yield was significant at 5% probability level but effect of hybrids and interaction effect of treatments was not significant (Table 2). Mean comparison effects of mixed fertilizer treatments indicated that the maximum and the minimum oil yield belonged to 50% chemical fertilizer with 100% bio-fertilizer (1.18 $t.ha^{-1}$) and 25% chemical fertilizer by 100% bio-fertilizer (0.95 $t.ha^{-1}$) (Table 3). Some researchers such as Mobasser and Moradgholi (2012) reported same result.

Chlorophyll index (SPAD)

Result of analysis of variance showed that the effect of different level of fertilizer on chlorophyll index was significant at 5% probability level but effect of hybrids and interaction effect of treatments was not significant (Table 2). Mean comparison effects of mixed fertilizer treatments indicated that the maximum and the minimum chlorophyll index belonged to 50% chemical fertilizer with 100% bio-fertilizer (53.04) and 25% chemical fertilizer by 100% bio-fertilizer (49.09) (Table 3). Amanolahi-Baharvand *et al.* (2014) reported the integrated fertilizer (50% urea and 50% vermicompost) management improved corn growth, chlorophyll content and remobilization in corn plants. Soleimanzadeh and Ghooshchi (2013) reported bio-fertilizer had significantly effects on the leaf chlorophyll, because inoculation with mycorrhiza increased the leaf chlorophyll (2.66 $mg.g^{-1}$ FW).

Correlation between traits

Evaluation result of relationship between measured traits indicated seed yield had significant and positive correlation with the biological yield (0.71**), harvest index (0.69**), oil yield (0.55*) and protein yield (0.51*) (Table 5). Some researchers such as the Szulc (2013) and Fahramand and Mobasser (2013) reported same results.

CONCLUSION

According to the results, growth promoting bacteria have positive role in the production of bio-fertilizers and hormones that play a significant role in regulating plant growth while mixing them with chemical fertilizers as a supplement the level and depth of the roots. This combination also increases the rate of water and nutrient absorbance which raise the rate of growth and photosynthesis. These combination also increase the seed yield, it has been found that bio-fertilizers can be combined with chemical fertilizers in a complementary way to reduce the excessive amount of chemical fertilizers used to grow corn. Finally consummation of biological fertilizers with 50% organic fertilizer produced the highest seed yield.

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